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(54) Method for stabilizing immunologically active substances immobilized on an insoluble carrier and their use in the preparation of reagents for measuring physiologically active substances.

(57) An immuno active substance immobilized on a carrier and stabilized by immersing said carrier in a solution of at least one of sugars and proteins can be used for measuring a physiologically active substance even after stored for a long period of time.

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*immobilizing
fixing sugar to solid
support
(minwell)*

STABILIZING METHOD OF IMMUNO ACTIVE SUBSTANCE
IMMOBILIZED ON INSOLUBLE CARRIER AND
ITS USE IN PREPARATION OF REAGENT
FOR MEASURING PHYSIOLOGICALLY ACTIVE SUBSTANCE

1 This invention relates to a process for stabilizing
immuno active substances immobilized on an insoluble carrier
and preparation of reagents for measuring a physiologically
active substance utilizing the immuno active substances
5 stabilized by the above process as their components.


Antigen-antibody reactions have been used for
measuring or detecting various physiologically active sub-
stances due to their high specificity and high sensitivity.
Specifically, radioimmunoassay (hereinafter referred to as
10 "RIA") systems have been applied to measure trace substances
(e.g., hormones such as insulin, glucagon, thyroxine, etc.;
high-molecular weight physiologically active substances
such as immunoglobulin E (Ig E), α -fetoprotein, CEA (carcino-
embrionic antigen), etc.) in biological samples such as
15 serum, urine, and tissue fluid, since RIA particularly
allows highly sensitive measurement. But it is also true
that the prevalence of RIA is limited due to some disadvant-
age. Reagents used in RIA are expensive and often unstable.
Complicated and expensive apparatuses are required for
20 reading the any results. Most of all, special safety stand-
ards are required to be get for handling of radioisotopes
and disposal of radioactive wastes.

On the other hand, the enzyme immunoassay (herein-
after referred to as EIA) was introduced in 1971 in order to
25 overcome these disadvantages in RIA. In EIA, an enzyme

1 is used as labeling substance instead of radioactive isotope.
An enzyme labeled reagent is inexpensive and stable for
a long period of time. EIA has the equivalent or higher
sensitivity for measurement as RIA. Further the test results
5 can be measured by the naked eye or a simple apparatus. Due
to such advantages over RIA, application of EIA is rapidly
expanded. But RIA and EIA are based on the same measuring
principles and only differ in their labeling substances.
As to measuring systems, there have been reported various
10 kinds of measuring systems, which can be divided into two
groups, that is, the heterogeneous measuring system which
employs the B/F separating method and the homogeneous measur-
ing system which does not employ the B/F separating method.
The B/F separating method indicates that a bound form of
15 an antigen and an antibody as a result of antigen-antibody
reaction (bound type, B) and a free form of an antigen and
antibody (free type, F) are physically separated. Homogene-
ous system depends on inhibition or activation of the enzyme
by one of the components (mainly antibody) after antigen-
20 antibody reaction. Since few cases of such enzyme-hapten
complex have been reported, the application of the homogene-
ous system is limited. Therefore, most present RIA and EIA
employ the heterogeneous measuring system. In the heterogene-
ous measuring system, a solid phase method wherein an antigen
25 or an antibody is immobilized on a water-insoluble carrier
has been most frequently employed for the B/F separation.
Although natural high-molecular compounds such as cellulose,
Sephacrose, agarose and dextran have been used as the water-

1 insoluble carrier, these compounds require much time for
washing procedure and centrifugational procedure, which
results in becoming a major factor for causing scattering
of measured values. In order to overcome these problems,
5 inorganic materials such as glass and synthetic polymers
such as polystyrene, polypropylene, poly(vinyl chloride)
are recently used as a carrier in the form of tubes, beads,
disks, fine particles (latex particles), microplates. By
using these materials as carrier, the centrifugational
10 procedure becomes unnecessary, and the washing procedure can
be simplified remarkably. Thus, reproducibility of measured
values becomes good, and employment of automated system
becomes possible and is actually practiced in some assay
fields. By the reasons mentioned above, establishment of
15 useful assay system in the heterogeneous measuring system
using the solid phase method much depends on a quality of
carrier.

Preferable properties of the carrier are as
follows:

- 20 (1) When an immuno active substance is bound to the
carrier, it should retain the immunological activity.
- (2) The carrier has no non-specific adsorption of
components included in a test sample.
- (3) The carrier has properties of binding strongly
25 with an immuno active substance.
- (4) The carrier has such properties as a surface
structure which makes binding with a sufficient amount of
immuno active substance possible.
- 

1 (5) Handling such as washing procedure accompanied
in the B/F separating procedure is simple and easy.

 In order to prepare a carrier which satisfies
the properties mentioned above and on which an immuno active
5 substance is attached, not only the selection of kind of
carrier but also the binding method of immuno active sub-
stance and the storing method of the carrier bound substance
are subject matters for development. In RIA and EIA employ-
ing the solid phase method, an immuno active substance is
10 immobilized on a carrier such as glass beads, polystyrene
beads, by covalent bond or physical adsorption method, and
the carrier bound substance is stored in a buffer solution
containing serum albumin.

 But such a method has many problems in that (i)
15 it is necessary to extract the buffer solution using
a filter paper at the time of use, which results in requiring
much labor and causing scattering of measured values, (ii)
when dried, deterioration of the solid phase takes place
due to decrease of the immunological activity of immuno
20 active substance, and (iii) there are many technical
problems for designing an automated assay system.

 It is an object of this invention to provide a
process for stabilizing an immuno active substance immobiliz-
ed on a carrier overcoming the disadvantages mentioned above,
25 and to provide a reagent utilizing the immuno active
substance thus stabilized on a carrier as its component
for measuring physiologically active substances.

 This invention provides a process for stabilizing

1 an immuno active substance immobilized on a carrier, which
comprises immersing a carrier bound an immuno active
substance in a solution of at least one member selected
from the group consisting of a sugar and a protein.

5 This invention also provides an assay reagent
for measuring a physiologically active substance comprising
as a component an immuno active substance immobilized on
a carrier and stabilized by immersing the carrier bound the
immuno active substance in a solution of at least one member
10 selected from the group consisting of a sugar and a protein.

As the carrier, there can be used any conventional
insoluble ones usually used in RIA and EIA. Preferable
carriers are insoluble (water-insoluble) ones which allow
easy solid-liquid separation without conducting centrifuga-
15 tional separation. Examples of such insoluble carriers
are synthetic polymer compounds such as polystyrene, poly-
propylene, poly(vinyl chloride), polyethylene, polychloro-
carbonate, silicone resin, silicone rubber, etc.; inorganic
materials such as porous glass, ground glass, alumina,
20 silica gel, activated charcoal, metal oxides, etc. These
materials can be used in any forms of tubes, beads, disk
flakes, fine particles (latex particles), microplates, etc.

As a method for immobilizing the immuno active
substance on the carrier, there can be used conventional
25 methods such as a covalent coupling method and a physical
adsorption method.

The covalent coupling method is a method for
fixing an immuno active substance on a water-insoluble



1 carrier by covalent bond. The largest number of reports
deal with this covalent coupling method among carrier binding
methods. The functional groups which participate the
binding of the immuno active substance with the carrier
5 are an α - or ϵ -amino group, an α -, β - or γ -carboxyl group,
a sulfhydryl group, a hydroxyl group, an imidazole group,
a phenol group, and the like. These functional groups react
with a diazonium group, an acid azide, an isocyanate or
an activated halogenated alkane. Therefore, by using such
10 a reactive functional group, it becomes possible to bind the
immuno active substance with the water-insoluble carrier by
covalent bond (e.g. see Taisha vol. 8, page 696, 1971). In
the case of using an inorganic material such as glass, the
inorganic material is first treated with a trialkoxysilane
15 derivative having a functional group such as γ -aminopropyl-
triethoxysilane in order to introduce a reactive functional
group thereinto. The resulting amino group-containing
alkylated glass thus obtained can be bound with an immuno
active substance by covalent bond by the same treatment as
20 in the case of amino group-containing immuno active sub-
stance. In general, introduction of a reactive aldehyde
group by the treatment with glutaraldehyde has been widely
used to couple an immuno active substance with a carrier
(J. Biochem., vol. 80, p. 895, 1976). There can also be
25 used various crosslinking agents depending on the kinds of
immuno active substances. For example, there can be used
succinaldehyde, malonaldehyde, or the like in addition to
glutaraldehyde mentioned above for crosslinking an amino

1 group with an amino group, m-maleimidobenzoyl-N-hydroxy-
succinimide ester, 4-(maleimidomethyl)cyclohexane-1-
carboxyl-N-hydroxysuccinimide ester for crosslinking an
amino group with a sulfhydryl group, and o-phenylenedi-
5 maleimide for binding a sulfhydryl group with a sulfhydryl
group.

The physical adsorption method is a method for
immobilizing an immuno active substance on a water-insoluble
carrier by physical adsorption. As the carrier, there can
10 be used inorganic materials such as activated charcoal,
porous glass, glass beads, alumina, a metal oxide, silica
gel, hydroxy apatite, etc.; and synthetic polymer compounds
such as polystyrene, polyethylene, poly(vinyl chloride),
polypropylene, polychlorocarbonate, etc. Among them, the
15 use of glass, polystyrene, or poly(vinyl chloride) in the
form of tubes, beads, disk flakes, fine particles (latex
particles), microplates are preferred.

As the immuno active substance to be immobilized
on the carrier, there can be used an antigen, an antibody
20 and a hapten (drugs, etc.).

Examples of the antigen are hormones such as
insulin, glucagon, growth hormone, human chorionic
gonadotropin, adrenocortical hormone, thyroid stimulating
hormone, etc.; proteins such as IgG, IgM, IgA, IgE, IgD,
25 α -fetoprotein, ferritin, β_2 -microglobulin, CEA, etc.;
and virus antigens such as HB_s antigen, rubella virus
antigen, etc.

Examples of the antibody are those obtained by



1 immunizing a mammal such as a rabbit, a guinea pig, a mouse,
a goat, a sheep or the like, or a bird such as a chicken,
a duck, or the like with an antigen or a hapten mentioned
below by a conventional method (e.g., antiinsulin antibody,
5 antiglucagon antibody, anti-IgG antibody, anti- α -fetoprotein
antibody, anti- β_2 -microglobulin antibody, etc.).

Examples of the hapten are steroid hormones,
catecholamines, and vitamins.

As the sugar solution, there can be used a solution
10 obtained by dissolving a monosaccharide such as ribose,
glucose, fructose, mannose, galactose, maltose, lactose,
sucrose, or the like, an oligosaccharide, or a polysaccharide
such as dextran, dextrin, or the like, these saccharides
being used alone or as a mixture thereof, in purified water
15 or a buffer solution. Among these sugar solutions, lactose,
sucrose, and dextrin solutions are preferred.

As the protein solution, there can be used a
solution obtained by dissolving a serum albumin such as a
bovine serum albumin, a human serum albumin, a sheep serum
20 albumin, or water-soluble gelatin, in purified water or a
buffer solution. Among these protein solutions, bovine
serum albumine and water-soluble gelatin solutions are
preferred.

The sugar solution and the protein solution can
25 be used alone or as a mixture thereof. When the mixed
solution of sugar and protein is used, more excellent effects
can be expected.

The sugar content in the sugar solution is

1 usually 0.1 to 10 weight/volume percent, preferably 2.5 to
5 weight/volume percent.

The protein content in the protein solution is
usually 0.1 to 2 weight/volume percent, preferably 0.5 to
5 1.5 weight/volume percent.

When the solution contains both sugar and protein,
the sugar content is usually 0.1 to 10 weight/volume percent,
and preferably 2.5 to 5 weight/volume percent and the
protein content is usually 0.1 to 2 weight/volume percent,
10 and preferably 0.5 to 1.5 weight/volume percent.

As the solvent for dissolving a sugar and/or a
protein, there can be used purified water or a buffer solu-
tion. Examples of the buffer solution are those having
buffering effect at near neutral pH such as a phosphate
15 buffer solution, a tris-HCl buffer solution, a Good's buffer
solution, and the like. Among them, the phosphate buffer
solution is particularly preferred. The molar concentration
of the buffer solution is usually 0.01 to 0.2 M, preferably
0.02 to 0.05 M and the pH of it is preferably 6.8 to 7.2.

20 When preparing the solution of sugar and/or
protein, there is no limitation to the order of addition
of these materials.

In order to stabilize the immuno active material
immobilized on a carrier in the dried state, the carrier
25 attaching the immuno active material is first immersed in
the solution of protein and/or sugar, for example, for 20
to 40 minutes at room temperature, and dried, for example,
by placing the thus treated carrier on a filter paper for

1 a sufficient time to allow air drying. The dried carrier
with stabilized immuno active substance can be used as a
reagent. More preferably, the thus dried carrier is
stored in a vessel sealed and capped under nitrogen gas
5 or reduced pressure. By subjecting the carrier to immers-
ing treatment in the solution of protein and/or sugar,
decrease of the antigen or antibody activity of immuno
active substance caused during air drying procedure of the
carrier can be prevented effectively.

10 The stabilized immuno active substances immobilized
on a carrier is useful as a reagent for measuring physiolog-
ically active substances in RIA or EIA.

Typical measuring systems in solid phase RIA and
EIA are a competitive method and a sandwich method.

15 The competitive method is based on the competitive
reaction between an unknown amount of the antigen in a
test sample and known amount of the same radioisotopically
or enzymatically labelled antigen to its antibody immobiliz-
ed on the solid phase. Amount of the antigen in a test
20 sample is quantified by measuring the solid phase bound or
unbound amount of radioactivity or enzymatic activity of
the labelled antigen.

On the other hand, the sandwich method is based
on the reaction that two specific antibodies sandwich an
25 unknown antigen to be measured. One of the antibodies is
immobilized onto a solid phase and the other is labelled
by a radioisotope or an enzyme. The amount of the antigen
to be measured is quantified by measuring the bound amount

1 of radioactivity or enzymatic activity of antibody on the solid phase.

Needless to say, the application of the present invention is not limited to the typical measuring systems
5 in RIA and EIA mentioned above. It also can be applied to various modified systems which utilize the immuno active substance immobilized on a carrier.

This invention is illustrated in detail by way of the following Examples, wherein all percents are by weight
10 unless otherwise specified.

Reference Example 1

(1) Preparation of Antiinsulin Antibody-Bound Glass Beads

Commercially available glass beads (6 - 7 mm in diameter) (500 pieces) were washed with purified water,
15 followed by washing with acetone. Then the glass beads were immersed in a 2% γ -aminotriethoxysilane/acetone solution and stood for 3 hours at room temperature. After the reaction, the glass beads were washed with acetone and purified water successively. The amino group-containing glass beads
20 thus obtained were activated by immersing them in a 25% glutaraldehyde solution for 2 hours at room temperature. After extensively washed with purified water, the glass beads were immersed in 100 ml of 0.02 M phosphate buffer (pH 7.3) containing 3 mg of guinea pig antiinsulin antibody
25 and allowed to stand at 4°C for 16 hours to bind the anti-insulin antibody to the glass beads. After the coupling reaction, the glass beads were washed with a 0.02 M phosphate

1 buffer (pH 7.3), and stored in a phosphate buffer (pH 7.3) containing 0.15M NaCl, 1% bovine serum albumin, 1 mM EDTA (ethylenediaminetetraacetic acid) and 0.05% NaN₃ in a cold place until the use.

5 (2) Preparation of Anti- β_2 -microglobulin Antibody-Bound Polystyrene Beads

Commercially available polystyrene beads (6.5 mm in diameter) (500 pieces) were washed with a 0.02 M phosphate buffer (pH 7.5) and then immersed in 100 ml of a 0.02 M
10 phosphate buffer (pH 7.5) containing 3 mg of rabbit anti- β_2 -microglobulin antibody and allowed to stand at 4°C for 16 hours to bind the anti- β_2 -microglobulin antibody to the polystyrene beads. After the reaction, the polystyrene beads were washed with a 0.02 M phosphate buffer (pH 7.3), stored
15 in a 0.02 M phosphate buffer (pH 7.3) containing 0.15 M NaCl, 1% bovine serum albumin, 1 mM EDTA and 0.05% NaN₃ in a cold place until the use.

(3) Preparation of Anti-C-Reactive Protein (C-RP) Antibody-Bound Poly(vinyl chloride) Plates

20 To each well of commercially available poly(vinyl chloride) microplates (U type, 96 wells), 0.1 ml of a 0.05 M carbonate buffer (pH 9.6) containing 5 μ g of mouse anti-C-RP antibody was added and allowed to stand at 4°C for 21 hours to bind the anti-C-RP antibody to the microplates. After
25 the reaction, each well was washed with a 0.01 M phosphate buffer (pH 7.4) containing 0.05% polyoxyethylene sorbitan monolauryl ether (Tween 20, a trade name, manufactured by Kao-Atlas Co., Ltd.), added with 0.2 ml of a 0.01 M phosphate

1 buffer (pH 7.4) containing 1% bovine serum albumin, allowed
to stand at 4°C for 19 hours, and stored in a cold place
until the use.

(4) Preparation of Anti-CEA Antibody-Bound Glass Beads

5 Commercially available 500 glass beads (6 - 7 mm
in diameter) were washed with purified water, followed by
washing with acetone. Then the glass beads were immersed
in a 2% γ -aminotriethoxysilane/acetone solution and stood
for 3 hours at room temperature. After the reaction, the
10 glass beads were washed with acetone and purified water
successively. The glass beads thus obtained were activated
by immersing in a 25% glutaraldehyde solution for 2 hours
at room temperature. After extensively washed with purified
water, the glass beads were immersed in 100 ml of 0.02 M
15 phosphate buffer (pH 7.3) containing 3 mg of rabbit antibody
and stood for 16 hours at 4°C to bind the anti CEA antibody
to the glass beads. After the coupling reaction, the glass
beads were washed with 0.02 M phosphate buffer (pH 7.3)
and stored in 0.02 M phosphate buffer (pH 7.3) containing
20 0.15 M NaCl, 1% bovine serum albumin, 1 mM EDTA and 0.05%
 NaN_3 in a cold place until the use.

Reference Example 2

[1] Measurement of Insulin by EIA Method Using Antiinsulin
Antibody-Bound Glass Beads

25 Measurement of Insulin by EIA

Reagents:

(1) Antiinsulin antibody-bound glass beads obtained in

- 1 Reference Example 1.
 - (2) Standard insulin of 0 to 320 μ U/ml
 - (3) Peroxidase labeled antiinsulin antibody.
 - (4) A 0.02 M phosphate buffer (pH 6.9) containing
- 5 0.15 M NaCl, 1% bovine serum albumin, 1 mM EDTA and 0.1% sodium salicylate for diluting the above-mentioned reagents (2) and (3).
 - (5) 60 mg of o-phenylenediamine.
 - (6) 1.7 v/v% hydrogen peroxide solution.
- 10 (7) A 0.05 M citrate-0.1 M phosphate buffer (pH 4.8) for dissolving the enzyme substrates of (5) and (6) mentioned above.
 - (8) 1.5 N H_2SO_4 .
 - (9) A color developing reagent solution in an amount of
- 15 20 ml containing 60 mg of o-phenylenediamine and 200 μ l of hydrogen peroxide obtained by dissolving the above-mentioned (5) and (6) in (7).

Assay Procedures:

- To 500 μ l of the reagent (3) diluted with the
- 20 reagent (4), 50 μ l of standard insulin solution was added, followed by addition of the reagent (1) to conduct the reaction at 37°C for 60 minutes. After the reaction, the beads were washed with 0.9% NaCl, followed by the addition of 500 μ l of the reagent (9) to start the enzymatic reaction.
 - 25 After incubating at 37°C for 15 minutes, 3.0 ml of the reagent (8) was added to stop the reaction and absorbance of the reaction mixture was measured at 492 nm.

- 1 [2] Measurement of β_2 -Microglobulin by EIA Method Using
Anti- β_2 -microglobulin Antibody-Bound Polystyrene Beads
Measurement of β_2 -Microglobulin by EIA

Reagents:

- 5 (1) Anti- β_2 -microglobulin antibody-bound polystyrene
beads obtained in Reference Example 1.
- (2) Standard β_2 -microglobulin of 0 to 200 $\mu\text{g}/\text{l}$.
- (3) Peroxidase labeled anti- β_2 -microglobulin antibody.
- (4) A 0.02 M phosphate buffer (pH 6.9) containing
- 10 0.15 M NaCl, 1% bovine serum albumin, 1 mM EDTA,
and 0.1% sodium salicylate for diluting the above-mention-
ed reagents (2) and (3).
- (5) 60 mg of o-phenylenediamine.
- (6) 1.7 v/v% hydrogen peroxide solution.
- 15 (7) A 0.05 M citrate-0.1 M phosphate buffer (pH 4.8)
for dissolving the enzyme substrates of (5) and (6) mentioned
above.
- (8) 1.5 N H_2SO_4 .
- (9) A color developing reagent solution in an amount
- 20 of 20 ml containing 60 mg of o-phenylenediamine and 200 μl of
hydrogen peroxide obtained by dissolving the above-mentioned
(5) and (6) in (7).

Assay Procedures:

- To 1 ml of the reagent (3) diluted with the re-
- 25 agent (4), 20 μl of standard β_2 -microglobulin was added,
followed by addition of the reagent (1) to conduct the
reaction at 37°C for 60 minutes. After the reaction,

- 1 the beads were washed with 0.9% NaCl, followed by the
addition of 500 μ l of the reagent (9) to start the enzymatic
reaction. After incubating at 37°C for 15 minutes, 3.0 ml
of the reagent (8) was added to stop the reaction and
5 absorbance of the reaction mixture was measured at 492 nm.

[3] Measurement of C-RP by EIA Method Using Anti-C-RP
Antibody-Bound Poly(vinyl chloride) Plates

Measurement of C-RP by EIA

Reagents:

- 10 (1) Anti-C-RP antibody-bound poly(vinyl chloride) plates
obtained in Reference Example 1.
- (2) Standard C-RP of 0 to 1000 ng/ml.
- (3) Peroxidase labeled anti-C-RP antibody.
- (4) A 0.02 M phosphate buffer (pH 7.3) containing 1%
- 15 bovine serum albumin, 0.5% polyoxyethylene nonylphenyl
ether (Nonipol 300, a trade name, manufactured by Sanyo
Chemical Industries, Ltd.) and 0.9% NaCl for diluting
the above-mentioned reagents (2) and (3).
- (5) 60 mg of o-phenylenediamine.
- 20 (6) 1.7 v/v% hydrogen peroxide solution.
- (7) A 0.05 M citrate-0.1 M phosphate buffer (pH 4.8)
for dissolving the enzyme substrates of (5) and (6) mentioned
above.
- (8) 6N H₂SO₄.
- 25 (9) A color developing reagent solution in an amount
of 20 ml containing 60 mg of o-phenylenediamine and 200 μ l
of hydrogen peroxide obtained by dissolving the above-

1 mentioned (5) and (6) in (7).

Assay Procedures:

To each well, 100 μ l of standard C-RP diluted
with the reagent (4) was added and allowed to stand at 37°C
5 for 120 minutes. Then, the reaction solution was removed
by suction and each well was washed with the reagent (4)
extensively. After adding 100 μ l of the reagent (3), the
reaction was conducted at 37°C for 120 minutes. After the
reaction, each well was washed with the reagent (4), followed
10 by addition of 100 μ l of the reagent (9) to start the
enzymatic reaction. After incubating at room temperature for
15 minutes, 50 μ l of the reagent (8) was added to stop the
reaction and absorbance of the reaction mixture was measured
at 490 nm by using a colorimeter for microplates.

15 [4] Measurement of CEA by EIA

Reagents:

- (1) Anti CEA antibody-bound glass beads obtained in
Reference Example 1.
- (2) Standard CEA of 60 μ g/ml.
- 20 (3) Peroxidase labeled anti CEA antibody.
- (4) A 0.02 M phosphate buffer (pH 7.0) containing 0.15 M
NaCl, 1% bovine serum albumin, 1 mM EDTA and 0.1% sodium
salicylate for diluting the above-mentioned reagents (2)
and (3).
- 25 (5) 60 mg of o-phenylenediamine.
- (6) 1.7 v/v% hydrogen peroxide solution

1 (7) A 0.05 M citrate-0.1 M phosphate buffer (pH 4.8)
for dissolving the enzyme substrates of (5) and (6) mentioned above.

(8) 1.5 N H_2SO_4 .

5 (9) A color developing reagent solution in an amount of 20 ml containing 60 mg of o-phenylenediamine and 200 μ l of 1.7% hydrogen peroxide obtained by dissolving the above mentioned (5) and (6) in (7).

Assay procedures:

To 500 μ l of the reagent (3) diluted with the
10 reagent (4), 50 μ l of standard CEA solution was added, followed by addition of the reagent (1) to conduct the reaction at 37°C for 18 hours. After the reaction, the beads were washed with 0.9% NaCl followed by the addition of 500 μ l of the reagent (9) to start the enzymatic reaction. After
15 incubating at 37°C for 30 minutes, 3 ml of the reagent (8) was added to stop the reaction and absorbance of the reaction mixture was measured at 492 nm.

Example 1

Stabilization of Antiinsulin Antibody-Bound Glass Beads

20 After washing the antiinsulin antibody-bound glass beads prepared in Reference Example 1 with purified water, the glass beads were immersed in the following treating solutions (a) to (e) at room temperature for 30 to 40 minutes.

25 (a) A 0.02 M phosphate buffer (pH 6.9) containing

1 5 w/v% sucrose and 1% bovine serum albumin.

(b) A 0.02 M phosphate buffer (pH 6.9) containing
5 w/v% sucrose.

(c) A 0.02 M phosphate buffer (pH 6.9) containing
5 1% bovine serum albumin.

(d) A 0.02 M phosphate buffer (pH 6.9) containing 1%
water-soluble gelatin.

(e) A 0.02 M phosphate buffer (pH 6.9).

After the treatment, the glass beads were air dried
10 at room temperature.

The glass beads thus obtained were subjected to
a severe test by storing the glass beads in a constant
temperature chamber at 40°C. Stability of the antibody-bound
glass beads were evaluated as follows. A sample containing
15 320 μ U/ml of insulin was measured by EIA method described
in Reference Example 2 and stability of the glass beads
was evaluated in terms of activity retention rate (%)
compared with the measured value obtained by using control
glass beads. The control glass beads were prepared as
20 described in Reference Example 1 and stored at 4°C in the
immersed state.

The results were shown in Table 1.

Table 1

Treating solution	Activity retention rate (%)	
	Stored for 2 weeks	Stored for 4 weeks
Control	100	100
(a)	94	89
(b)	70	68
(c)	47	19
(d)	71	66
(e)	14	9

1 Example 2

Stabilization of Anti- β_2 -microglobulin Antibody-Bound Polystyrene Beads

The anti- β_2 -microglobulin antibody-bound polystyrene
5 beads prepared in Reference Example 1 were immersed in purified water. After removing water on a filter paper, the polystyrene beads were immersed in the following treating solutions (a) to (e) at room temperature for 30 to 40 minutes.

(a) A 0.02 M phosphate buffer (pH 6.9) containing
10 5 w/v% sucrose and 1% bovine serum albumin.

(b) A 0.02 M phosphate buffer (pH 6.9) containing
5 w/v% sucrose.

(c) A 0.02 M phosphate buffer (pH 6.9) containing
1% bovine serum albumin.

15 (d) A 0.02 M phosphate buffer (pH 6.9) containing

1 1% water-soluble gelatin.

(e) A 0.02 M phosphate buffer (pH 6.9).

After the treatment, the polystyrene beads were taken out from the solutions and placed on a filter paper
5 to remove the water and air dried at room temperature.

The polystyrene beads thus treated were subjected to the severe test in the same manner as described in Example 1 by storing them in the constant temperature chamber at 40°C. Stability of the antibody-bound polystyrene beads were
10 evaluated as follows. A sample containing 200 µg/l of β_2 -microglobulin was measured by EIA method described in Reference Example 2 and evaluated in terms of activity retention rate (%) compared with the measured value obtained by using control polystyrene beads. The control polystyrene
15 beads were prepared as described in Reference Example 1 and stored at 4°C in the immersed state.

The results were shown in Table 2.

Table 2

Treating solution	Activity retention rate (%)	
	Stored for 2 weeks	Stored for 4 weeks
Control	100	100
(a)	102	101
(b)	93	81
(c)	81	42
(d)	80	79
(e)	42	37

1 Example 3

Stabilization of Anti-CRP Antibody-Bound Poly(vinyl chloride) Microplates

The anti-CRP antibody-bound poly(vinyl chloride) microplates prepared in Reference Example 1 were treated by using the following solutions and procedures.

(a) 4% Lactose solution was poured into each well of microplates and then each well was dried.

(b) 0.02 M Hepes buffer (pH 7.0) containing 1% bovine serum albumin was poured in each well of microplates and then each well was dried.

(c) 0.01 M Phosphate buffer (pH 7.4) containing 1% bovine serum albumin was poured in each well of microplates and stored in the poured state.

(d) Each well was air dried without treatment.



1 After the treatment, microplates were stored at
25°C for 7 weeks. Stability of the antibody-bound micro-
plates was evaluated as follows. A sample containing 1000
µg/ml CRP was measured by EIA method described in Reference
5 Example 2 and evaluated in terms of activity retention rate
(%) compared with the measured value obtained by control
microplates which were prepared in the same manner as
described in Reference Example 1 at the time of use.

The results were shown in Table 3.

Table 3

Treating procedures	Activity retention rate (%)
Control	100
(a)	95
(b)	90
(c)	89
(d)	0

10 Example 4

After washing the anti CEA antibody-bound glass
beads prepared in Reference Example 1 with purified water,
the glass beads were immersed in the following solutions
(a) to (h) at room temperature for 30 to 40 minutes.

15 (a) A 0.02 M phosphate buffer (pH 7.0) containing

1 5% sucrose and 1% bovine serum albumin

(b) A 0.02 M phosphate buffer (pH 7.0) containing
5% lactose.

(c) A 0.02 M tris-HCl buffer (pH 7.2) containing
5 5% mannose and 1.5% water-soluble gelatine.

(d) A 0.02 M tris-HCl buffer (pH 7.2) containing 4%
dextrin.

(e) A 0.02 M Hepes buffer (pH 7.2) containing 5%
sucrose.

10 (f) A 0.02 M phosphate buffer (pH 7.0).

(g) A 0.02 M tris-HCl buffer (pH 7.2).

(h) A 0.02 M Hepes buffer (pH 7.2).

After the treatment, the glass beads were air
dried at room temperature.

The glass beads thus obtained were subjected to
15 a severe test by storing them in a constant chamber at 40°C.
Stability of the antibody-bound glass beads were evaluated
as follows. A sample containing 60 µg/ml CEA was measured by
EIA method described in Reference Example 2. The stability of
the glass beads was evaluated in terms of activity retention
20 rate (%) compared with the measured value obtained by using
control glass beads. The control glass beads were prepared
as described in Reference Example 1 and stored at 4°C in the
immersed state.

The results were shown in Table 4.

Table 4

Treating solution	Activity retention rate (%)	
	Stored for 2 weeks	Stored for 4 weeks
Control	100	100
(a)	98	92
(b)	75	70
(c)	92	85
(d)	70	65
(e)	83	76
(f)	15	6
(g)	12	3
(h)	20	11

CLAIMS:

1. A process for stabilizing an immuno active substance immobilized on a carrier, which comprises immersing a carrier bound an immuno active substance in a solution of at least one member selected from the group consisting of a sugar and a protein.
2. A process according to Claim 1, which further comprises drying the immersed carrier.
3. A process according to Claim 1, wherein the carrier is a synthetic polymer material or an inorganic substance.
4. A process according to Claim 1, wherein the immuno active substance is an antigen.
5. A process according to Claim 1, wherein the immuno active substance is an antibody.
6. A reagent for measuring a physiologically active substance comprising as a component an immuno active substance immobilized on a carrier and stabilized by immersing the carrier bound the immuno active substance in a solution of at least one member selected from the group consisting of a sugar and a protein.
7. A reagent according to Claim 6, wherein the carrier is an inorganic substance.
8. A reagent according to Claim 6, wherein the carrier is a synthetic polymer material.
9. A reagent according to Claim 6, wherein the immuno active substance is an antigen.
10. Use of a reagent according to Claim 6 for measuring a physiologically active substance by enzyme immunoassay

or radioimmunoassay.

1



DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
P,X	GB-A-2 124 231 (CORNING GLASS WORKS) * Whole document *	1-10	G 01 N 33/543 G 01 N 33/545 G 01 N 33/551

X	GB-A-2 016 687 (ABBOTT LABORATORIES) * Whole document *	1-10	

X	EP-A-0 063 810 (CIBA-GEIGY AG.) * Claims 1,2,7,32,33 *	1-10	

X	EP-A-0 042 755 (UNILEVER NV.) * Claims 1,4; page 10, lines 11-16 *	1-10	

			TECHNICAL FIELDS SEARCHED (Int. Cl.4)
			G 01 N
The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 16-11-1984	Examiner GRIFFITH G.
CATEGORY OF CITED DOCUMENTS			
X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document	